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Terminal Deoxynucleotidyltransferase

SEROLOGICAL STUDIES AND RADIOIMMUNOASSAY*

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Mouse antisera against calf terminal deoxynucleotidyltransferase (terminal transferase) have been prepared. The sera have been used to characterize terminal transferase both by studying inhibition of enzyme activity and by developing a competition radioimmunoassay using highly purified ^{125}I-labeled terminal transferase. By either assay, anti-terminal transferase serum did not cross-react significantly with calf DNA polymerases α and β, *Escherichia coli* DNA polymerase I, or the reverse transcriptase of Moloney mouse leukemia virus. The calf terminal transferase did, however, share cross-reactive but not identical determinants with human and murine terminal transferase. The radioimmunoassay could detect as little as 2 ng of terminal transferase/mg of soluble protein in a tissue extract. Thymocytes were found to contain 280 ng of terminal transferase/mg of cell protein or about 1×10^5 molecules/cell; bone marrow had about 1% of the level of enzyme found in thymus. Extracts of spleen, peripheral white blood cells, lymph nodes, liver, muscle, and kidney all lacked detectable antigenicity of terminal transferase. These data indicate that terminal transferase is a tissue-specific enzyme and is not related to other DNA polymerases.

Terminal deoxynucleotidyltransferase (terminal transferase) is an enzyme with the unique ability to polymerize deoxynucleotides onto a primer in the absence of a template (1). It has been purified to homogeneity and extensively characterized (1, 2). In humans (3), rats (4), and mice (5, 6), terminal transferase activity appears to be restricted to the thymus and bone marrow cells among various tissues examined. The cells containing terminal transferase in the primary lymphoid organs have been partially characterized (3–6). The function of terminal transferase in the physiology of the thymus and bone marrow cells remains unknown, although it has been suggested that the enzyme might act as a somatic mutator in the generation of diversity (7).

Our interest in terminal transferase originated from the discovery of the enzyme in the circulating leukemic cells of a patient with acute lymphoblastic leukemia (8). As part of our effort to improve the method of detecting terminal transferase, we have now developed a radioimmunoassay for the enzyme. With this assay the enzyme can be detected in the presence of inhibitors and accurately quantitated independent of the vagaries of enzymatic assay. In this communication we describe the preparation and characterization of an antiserum made against calf terminal transferase. By using this antiserum and ^{125}I-labeled pure enzyme, a competition radioimmunoassay has been developed, and applied to study the antigenic relatedness of terminal transferase to other DNA polymerases and to quantitate terminal transferase in crude tissue extracts.

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EXPERIMENTAL PROCEDURE

Methods

Assay of DNA Polymerases—Eukaryotic DNA polymerases were named according to a system recently devised (9, 10). Templates, primers, and deoxynucleotide triphosphates were obtained from commercial sources (8). Standard reaction mixtures (100 μl) contained 0.05 M Tris-HCl (pH 8.3) and one of the following sets of reagents: (*a*) for terminal transferase assay: 2 μg of oligo(dA)/0.6 mM $MnCl_2$/2 mM dithiothreitol/450 pmol of [^{3}H]dGTP (2600 cpm/pmol); (*b*) for DNA polymerase α assay: 1 μg of poly(dC)/0.5 μg of poly(dI)/4 mM magnesium acetate/450 pmol of [^{3}H]dGTP (2600 cpm/pmol)/2 mM dithiothreitol; (*c*) for DNA polymerase β assay: 2 μg of poly(dA-dT)/ 6 mM magnesium acetate/2 mM dithiothreitol/400 pmol of [^{3}H]dTTP (2300 cpm/pmol)/ 20 nmol of dATP; (*d*) for DNA polymerase γ assay: 1 μg of poly(rA)/0.5 μg of oligo(dT)/0.8 mM manganese chloride/100 pmol of [^{3}H]dTTP (2000 cpm/pmol); (*e*) for reverse transcriptase assays: 2 μg of poly(rC)/0.4 μg of oligo(dG)/6 mM magnesium chloride/2 mM dithiothreitol/450 pmol of [^{3}H]dGTP (2200 cpm/pmol).

Enzyme Neutralization Assay—Reaction mixtures containing appropriate amounts of enzymes and antiserum in 70 μl of 0.1 M KCl/25 mM Tris (pH 8.3)/20% glycerol/500 μg/ml of bovine serum albumin were incubated at 37° for 20 min (Incubation I). Then 30 μl of a concentrated reaction mixture containing substrates, primers, templates, and metal ions optimal for each enzyme to be tested were added, and the reaction was allowed to proceed at 37° for another 20 min (Incubation II). The assays were terminated by adding an equal volume of 20% trichloroacetic acid.

Immunization—F_1(CBA/H-T6J × SJL/J) female mice (8 weeks old) were injected intraperitoneally with 2×10^9 heat-killed *Bordetella pertussis* organisms. Four hours later they were injected intraperitoneally with 30 μg of the A-2 terminal transferase (see "Materials"), which had been preadsorbed to alum according to a published procedure (11). Schedules of antigen administration and bleeding were as described in the legend to Fig. 1. Mice were bled from the tail vein into glass tubes. After 10 hours at 4°, antisera were collected by centrifuging the clotted blood at 1,000 × *g* for 5 min and then stored at −70°. The antisera were used without further purification. Unless otherwise stated, the serum used for the experiments described here was the fifth or sixth bleeding from Animal 1.

Purification of Terminal Transferase for Radioimmunoassay—Calf thymus glands (1.2 kg) with contaminating tissues removed were processed for terminal transferase purification according to the published method (2). The specific activity of the final purified enzyme was 4140 units/mg of protein, which represents at least a 4,000-fold purification from the crude thymic extracts (see Fig. 11). Over 98% of the protein of this preparation appears to be terminal transferase as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 5). The purified terminal transferase was stored in 0.3 M potassium phosphate buffer, pH 7.2, at −70°.

Iodination of Terminal Transferase—Protein iodination was carried out by the method of Greenwood *et al.* (12). Reaction mixtures (35 μl in 0.3 M potassium phosphate buffer, pH 7.4) containing 2 μg of purified terminal transferase, 500 μCi of $Na^{125}I$ (585 mCi/ml) and 0.2 μg of chloramine-T were incubated for 3 min in an ice bath. The iodinated calf terminal transferase was separated from free $Na^{125}I$ by gel chromatography on a Sephadex G-25 column (0.5 × 15 cm) equilibrated with 0.3 M potassium phosphate buffer, pH 7.2. The ^{125}I-terminal transferase was further purified on a Sephadex G-75 column (1.5 × 100 cm) equilibrated with 0.3 M potassium phosphate buffer, pH 7.2/10 μg/ml of bovine serum albumin/0.01% Triton X-100 (Buffer A).

The specific activity of ^{125}I-terminal transferase was 2.5 to 5 × 10^3 cpm/ng. The iodinated enzyme co-chromatographed with native calf terminal transferase on a Sephadex G-75 column, indicating no gross change in enzyme aggregation after its iodination.

Radioimmunoassay for Terminal Transferase—The mouse anti-terminal transferase was titrated as described by Panet *et al.* (13). Then 3 μl of normal mouse serum plus 5 μl of mouse anti-terminal transferase serum (diluted in Buffer A containing 1 mg/ml of bovine serum albumin) were added to tubes in quantity sufficient to precipitate about 50% of the input ^{125}I-terminal transferase. Additional components were added in the following sequence: the unknown sample, ^{125}I-terminal transferase (2 to 4 ng), goat anti-mouse IgG[1] (30 μl containing 2 mM EDTA). After each addition the reaction tube was incubated at 37° for 1 hour, then at 4° for 6 hours more. Final reaction volume was 230 μl. The final immunoprecipitate was collected by centrifugation at 3,000 × *g*, washed two times with Buffer A, and counted in a γ spectrometer. In competition radioimmunoassay 100% precipitation represents the amount of ^{125}I-terminal transferase precipitated in the absence of any competing antigen.

Preparation of Cellular Extract for Radioimmunoassay—Tissues in 4 volumes of Buffer B (0.2 M potassium phosphate, pH 7.2/1 mM EDTA/1 mM mercaptoethanol/5% ethanol/2 mM phenylmethylsulfonylfluoride) were homogenized in a Waring Blendor at high setting for 2 min at 4°. After adding Triton X-100 to a final concentration of 0.5%, the homogenates were stirred at 4° for another 2 hours, and then centrifuged at 10,000 × *g* for 10 min. The supernatant was extracted with 5 volumes of ether three times, and dialyzed three times against 100 volumes of Buffer C (0.3 M potassium phosphate, pH 7.2/0.01% Triton X-100/0.5 mM EDTA/1 mM mercaptoethanol). The extract was then centrifuged at 100,000 × *g* for 60 min and the supernatant was used for radioimmunoassay. When cells were used as the starting materials, they were homogenized in a tightly fitted Dounce glass homogenizer and processed in a manner identical to that described above.

Miscellaneous Methods—Amounts of protein were determined by the method of Lowry *et al.* (14) using bovine serum albumin as standard. Analyses by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were performed according to Laemmli (15).

Materials

Enzyme Preparations—Mouse and human terminal transferases were partially purified as described in earlier reports (3, 5). Moloney mouse leukemia virus reverse transcriptase was purified to homogeneity (16). Calf DNA polymerase α (12,400 units/mg, containing 10 to 30% enzyme protein), and calf DNA polymerase β (110,000 units/mg, containing 50% enzyme protein) were kindly provided by Dr. L. M. S. Chang, University of Connecticut Medical Center. *Escherichia coli* DNA polymerase I was a generous gift of Dr. A. Kornberg, Stanford University. Fresh calf thymus glands were purchased from Trelegan Co., Cambridge, Mass.

Antigen for Immunization—Two lots of calf terminal transferase (Lot A-2, 2117 units/mg; Lot 3363, 331 units/mg[2] purchased from the P-L Biochemical Inc., were screened for their use as antigen. The enzymes were prepared according to Chang and Bollum (2). Lot A-2 was further characterized because of its higher specific activity. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that A-2 protein contains two major bands corresponding to the two subunits of calf terminal transferase as described by Chang and Bollum (2). Later analysis of A-2 protein by the radioimmunoassay (Fig. 8) indicated that about 60% of its total protein was terminal transferase. No contaminating enzyme activities of DNA polymerase α, β, and γ were detected in Lot A-2. Because a monospecific antiserum is not a necessary requirement for the development of a radioimmunoassay, the A-2 protein was considered acceptable for use as an antigen for immunization.

Reagents—Goat anti-mouse IgG was a product of Meloy Laboratory, Springfield, Virginia. $Na^{125}I$ (585 mCi/ml, >14 mCi/μg) was purchased from Amersham/Searle, Arlington Heights, Ill. Sephadex G-25, G-75, and G-100 were obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N. J. Electrophoretically pure bovine serum albumin was purchased from Calbiochem, San Diego, Calif.

RESULTS

Immunization and Characterization of Antisera

To study the response of mice injected with terminal transferase, serum from injected mice was collected at various times and assayed for its ability to neutralize the enzymatic activity of terminal transferase. The immune response of two animals is shown in Fig. 1. Neutralizing activity in the sera could be detected 1 week after the second antigen injection. Activity was maximal by 12 days after the second injection and was maintained by a third injection. In mouse 2 the activity dropped markedly thereafter, while in mouse 1 it stayed at a high level for at least 100 days.

To determine whether the neutralizing activity of the serum was due to immunoglobulin, the ability of goat anti-mouse IgG to precipitate the inhibiting factor was tested. For this experiment, normal mouse serum and the mouse anti-terminal transferase serum were incubated with goat anti-mouse IgG and then centrifuged to remove the precipitate. The ability of the treated mouse sera to inhibit terminal transferase was then compared to that of sera that had not been treated with goat anti-mouse IgG (Table I). The goat serum by itself inhibited terminal transferase about 50%; the inhibition is presumably not due to anti-terminal transferase activity because the goat

[1] The abbreviation used is: IgG, immunoglobin G.

[2] One enzyme unit is the amount of enzyme that incorporates 1 nmol of dGMP onto an oligo(dA) primer during 1 hour at 37°. Enzyme assays for terminal transferase in this report, unless otherwise stated, were done in a Tris-buffered system as described in "Methods." In the literature, terminal transferase assay has often been carried out in a cacodylate-buffered system (0.2 M potassium cacodylate, pH 7.2; 4 mM $MgCl_2$; 1 mM 2-mercaptoethanol; 10 mM $d(pT)_3$; and 1 mM dATP; see Ref. 2). For the same amount of purified terminal transferase, a much higher enzyme activity can apparently be obtained in the cacodylate-buffered system than in the Tris-buffered system. For example, a batch of calf terminal transferase (the enzyme was a gift from Dr. Robert L. Ratliff of the Los Alamos Laboratory, New Mexico) which had a specific activity of 32,700 units/mg assayed in the cacodylate buffered system, turned out to be 1260.5 units/mg assayed in the Tris-buffered system. The ratio of the sensitivity of the two enzyme assays is therefore 26. We continue to use the Tris system because we have been thus far unable to reproduce the higher literature values using cacodylate.

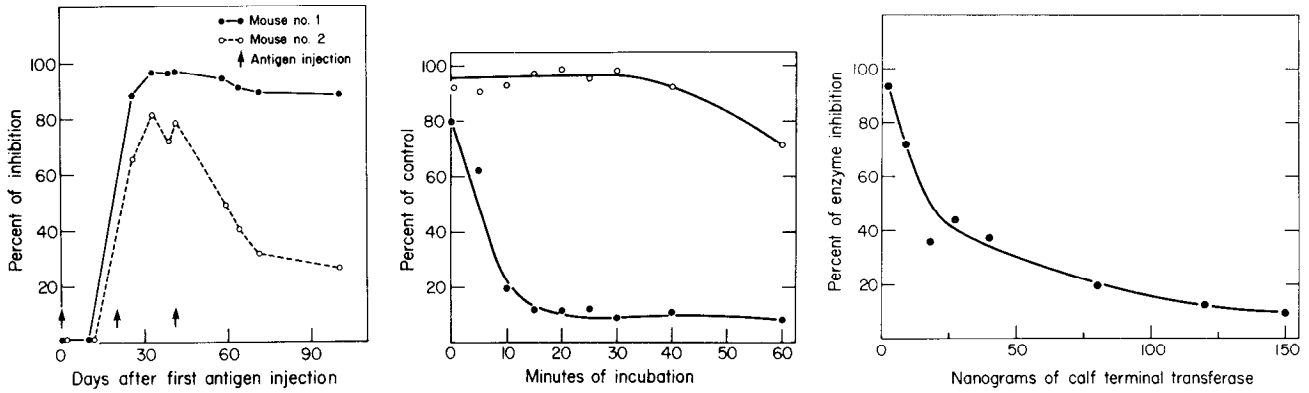

FIG. 1 (*left*). Immune response monitored by enzyme neutralization assay. Two animals were injected with terminal transferase at Day 0 and reinjected at the time indicated by the *arrows*. At various times the mice were bled and the anti-terminal transferase titer of the serum was studied. Portions of 0.12 unit (40 ng) of calf terminal transferase were incubated with antisera (final dilution 1/150). The neutralization assays were carried out as described under "Methods." ●——●, Mouse 1; O- - -O, Mouse 2.

FIG. 2 (*center*). Kinetics of enzyme neutralization by antiserum. Portions of 40 ng (0.12 unit) of calf terminal transferase were incubated at 37° with 31.2 μg of antiserum protein (final antiserum dilution 1/140) or an equivalent amount of normal mouse serum protein. Samples were withdrawn at desired intervals and frozen in acetone/dry ice bath. After the last time *point*, all samples were thawed and mixtures (30 μl) containing reaction reagents added. The enzymatic reaction was allowed to proceed at 37° for 20 min (see "Methods"). Only 20% of the terminal transferase activity was neutralized during the 20-min incubation for enzymatic assay (see zero time *point*). Enzyme activity measured in the absence of any serum protein was taken as 100%. ●——●, immune serum; O——O, normal serum.

FIG. 3 (*right*). Titration of fixed amounts of antiserum against increasing amounts of calf terminal transferase. Neutralization mixtures were established under "Methods," containing 4.2 μg of nonimmune or immune antiserum protein (1/100 final antiserum dilution) and serial dilutions of calf terminal transferase. The ratio of enzyme activity after treatment with immune or nonimmune serum was used to compute the percentage of enzyme inhibition.

TABLE I

Nature of Enzyme Neutralization Factor in Mouse Antiserum

Where indicated, mouse sera (2 μl in 18 μl of 140 mM NaCl/5 mM KCl/16 mM Na_2HPO_4/3 mM KH_2PO_4, pH 7.4) were incubated with or without 20 μl of goat anti-mouse IgG (53.8 mg/ml) at 37° for 1 hour, then at 4° overnight. At the end of incubation, reaction mixtures were centrifuged at 10,000 × *g* for 10 min. Supernatants (10 μl) were tested for neutralization activity against 0.11 unit of terminal transferase as described under "Methods."

Addition	Enzyme activity	Inhibition
	pmol dGMP incorporated	%
None	218	
Normal mouse serum	213.7	6.6
Mouse anti-terminal transferase	3.6	98.3
Goat anti-mouse IgG	104	52.3
Goat anti-mouse IgG plus normal mouse serum	108	50.5
Goat anti-mouse IgG plus mouse anti-terminal transferase	106.2	51.3

serum could not precipitate ^{125}I-terminal transferase (See Fig. 6). In spite of the inhibitory activity of the goat serum, its ability to precipitate the terminal transferase-neutralizing activity of the mouse antiserum is evident (Table I), and therefore the neutralizing factor must be immunoglobulin.

Properties of Enzyme Neutralization Reaction

The kinetics of enzyme neutralization by the mouse anti-terminal transferase serum are shown in Fig. 2. Inhibition was maximal after incubating terminal transferase with the mouse antiserum at 37° for 20 min.

A titration of increasing amounts of terminal transferase against fixed amounts of antisera is shown in Fig. 3. At 1/1000 dilution (4.2 μg of antiserum proteins), the more antigen added, the less inhibition was observed.

A titration of increasing amounts of immune sera against fixed amounts of terminal transferase is shown in Fig. 4 (Δ). The enzyme was preincubated with the antiserum prior to the determination of residual enzyme activity. The enzyme activity of 40 ng of terminal transferase was reduced to 50% of control at 5.4 μg of antiserum protein (1/800 antiserum dilution).

Specificity of Antiserum

By enzyme neutralization assay, the antiserum did not appear to significantly inhibit calf DNA polymerases α and β, *Escherichia coli* DNA polymerase I, or reverse transcriptase from Moloney mouse leukemia virus (Fig. 4). It did, however, inhibit human and, less extensively, murine terminal transferase. Even using less human or mouse terminal transferase than the amount of calf terminal transferase used, much more antiserum was needed to achieve the same degree of inhibition with heterologous terminal transferase than with the calf enzyme.

Radioimmunoassay

Preparation of Labeled Enzyme—For radioimmunoassay, the enzyme used for iodination should be of the highest purity. We prepared terminal transferase from calf thymus for this purpose (see "Methods"). The preparation had a specific activity of 4140 units/mg and represented at least a 4000-fold purification as determined by radioimmunoassay (see Fig. 11). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that greater than 95% of protein in this preparation was contained in the two subunits of terminal transferase (Fig. 5). The molecular weight of the large subunit is 28,000, and that of the small subunit is 8,000. After iodination, the majority of the ^{125}I was contained in the two terminal transferase bands (Fig. 5). Much of the ^{125}I not in the terminal transferase was not immunoprecipitable—the ^{125}I in the immunoprecipitate was virtually all in the terminal transferase bands (Fig. 5).

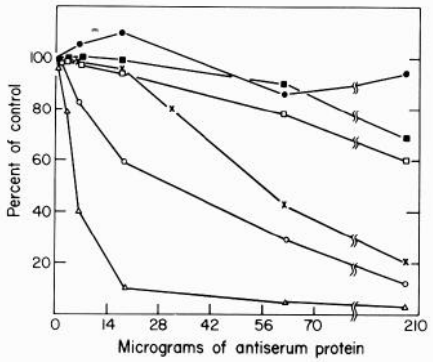

FIG. 4. Effect of increasing amounts of anti-terminal transferase serum on the activity of various DNA polymerases. Neutralization mixtures were established as described under "Methods." They contained either 0.06 unit of human or mouse terminal transferase, 0.12 unit of calf terminal transferase, 0.05 unit of calf DNA polymerase α and β, or 0.1 unit of Moloney mouse leukemia virus reverse transcriptase (final antiserum dilution, 1/800). The enzyme activity in the presence of immune protein is expressed as a percentage of the activity in the presence of identical amounts of nonimmune serum protein. Δ——Δ, calf terminal transferase; ○——○, human terminal transferase; × ——×, mouse terminal transferase; □——□, calf DNA polymerase α; ■——■, calf DNA polymerase β; ●——●, reverse transcriptase of Moloney mouse leukemia virus.

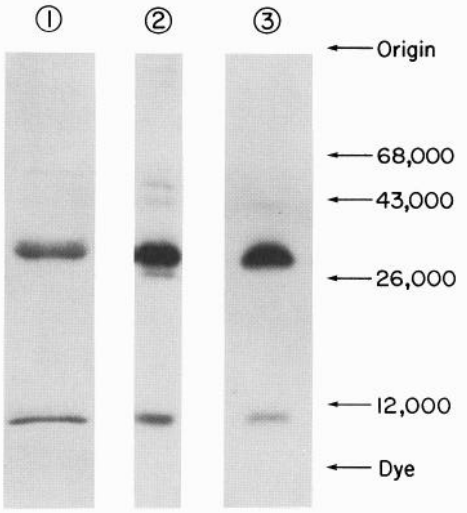

FIG. 5. Analysis of the purified calf terminal transferase by electrophoresis through sodium dodecyl sulfate-polyacrylamide gel. *1*, enzyme (20 μg) was submitted to electrophoresis and then stained with Coomassie blue. *2*, ^{125}I-terminal transferase (25,000 cpm) preparation was submitted to electrophoresis and then the gel was developed by autoradiography. *3*, ^{125}I-terminal transferase (22,000 cpm) was precipitated by antiserum as described under "Methods." The precipitate was submitted to electrophoresis and developed by autoradiography. Molecular weights of markers analyzed on a parallel gel: bovine serum albumin, 68,000; ovalbumin, 43,000; chymotrypsinogen, 26,000; cytochrome *c* 12,500. The concentrations of all three gels were 12.5%.

Immunoprecipitation of ^{125}I-terminal Transferase and Its Inhibition—The capacity of various antiserum dilutions to precipitate ^{125}I-terminal transferase is shown in Fig. 6. With high antiserum concentration, 90% of the labeled enzyme was immunoprecipitable (if the Sephadex G-75 column chromatography of the iodinated enzyme was omitted, 75 to 80% was precipitable). Preimmune serum from the same animal that produced the antiserum precipitated less than 2% of the labeled enzyme.

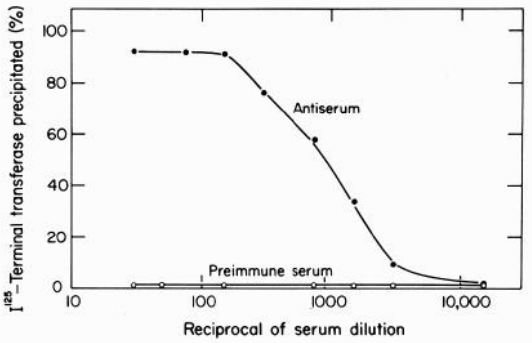

FIG. 6. Immunoprecipitation of ^{125}I-calf terminal transferase by varying concentrations of antiserum. Reaction was carried out essentially as described by Panet *et al.* (13) except using Buffer A. Either anti-terminal transferase serum (●——●) or the preimmune serum from the same mouse (○——○) were used. Input ^{125}I-terminal transferase, 7,000 cpm.

The immune response of the two mice to injected antigens was quantitated by radioimmunoassay (Fig. 7). It was very similar to that obtained by enzyme neutralization assay (compare to Fig. 1).

To establish a competition radioimmunoassay, from the data of Fig. 6 an amount of antiserum was determined that would precipitate 50% of the labeled enzyme. Competition of this precipitation was studied by incubating increasing amounts of unlabeled enzyme with the antiserum before using it to precipitate ^{125}I-terminal transferase. With this system, about 5 ng of terminal transferase could be detected, an amount of enzyme that displaced 10% of the labeled terminal transferase (Fig. 8).

Comparison of Enzyme Purity by Radioimmunoassay—The terminal transferase preparation used to elicit the mouse anti-terminal transferase serum was compared for purity with the highly purified terminal transferase used for radioiodination. To compete 20% of the ^{125}I-labeled terminal transferase required 8 ng of the highly pure terminal transferase and 13 ng of the preparation used as antigen (Fig. 8). This difference agrees well with the approximately 2-fold higher specific activity of the purer terminal transferase preparation and demonstrates the ability of radioimmunoassay to monitor enzyme purity.

Specificity of Assay—The ability of heterologous terminal transferase and other DNA polymerases to compete with the labeled calf terminal transferase was investigated. Calf terminal transferase did not appear to cross-react with *E. coli* DNA polymerase I, Moloney mouse leukemia virus reverse transcriptase, or calf thymus DNA polymerases α and β (Fig. 9). The preparation of calf DNA polymerase α was found to be contaminated with terminal transferase (0.5% of total protein) as determined by enzyme assay. The data presented here have been corrected for this contamination.

Human and murine terminal transferase did compete with the ^{125}I-terminal transferase (Fig. 10). The slopes of the radioimmunoassay competition curves for homologous and heterologous terminal transferase were quite distinct, however, indicating that the determinants recognized by the mouse antiserum on the calf enzyme are represented on human and mouse terminal transferase in a modified form. The apparent distant relatedness of murine terminal transferase with calf enzyme might be a result of the fact that the antiserum was elicited in the mouse.

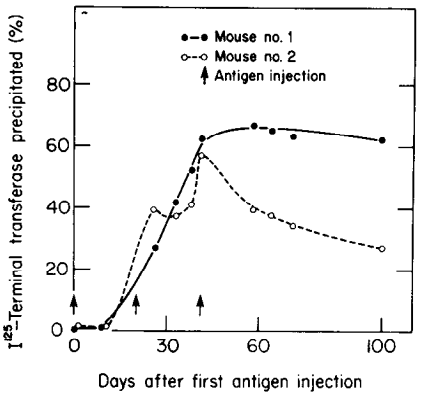

FIG. 7. Titer of the antisera determined by radioimmunoassay. Immunoprecipitation of ^{125}I-terminal transferase (7,000 cpm) was conducted as described under "Methods." The final dilution of all antisera tested was 1/750.

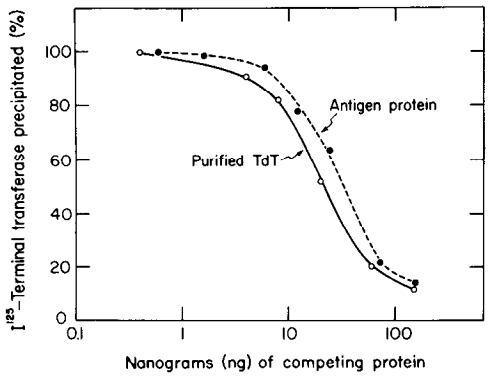

FIG. 8. Competition radioimmunoassay. Immunoprecipitation of ^{125}I-terminal transferase was competed by increasing amounts of either unlabeled calf terminal transferase (Lot A-2 used for immunization) (●- - -●) or purified terminal transferase (*TdT*) (○——○). The final concentration of the antiserum was 1/1000, which gave 45% precipitation of input ^{125}I-terminal transferase (6500 cpm).

Tissue Distribution of Terminal Transferase—The presence of terminal transferase among various calf tissues was studied by radioimmunoassay. As shown in Fig. 11, the antigenic determinants of terminal transferase could only be detected in the thymus and bone marrow. Comparing the curve for pure terminal transferase with the curve for thymus, we estimate about 280 ng of terminal transferase/mg of soluble cell protein. Bone marrow would have about 1% of the terminal transferase concentration of thymus. The terminal transferase concentration in negative tissues (spleen, lymph nodes, liver, muscle, white cells from peripheral blood, and kidney) must be lower than 2 ng of terminal transferase/mg of protein, or less than 0.7% as much as that of the thymus. Mixing extracts of negative tissues with thymus extracts produced no masking of the terminal transferase in thymus, indicating that the negative tissues truly lack terminal transferase.

DISCUSSION

The ability to elicit antibodies to terminal transferase has allowed us to study the antigenic sites on the enzyme using two different assays, inhibition of enzymatic activity and precipitation of labeled enzyme. The two types of assays give similar pictures: terminal transferase appears to be a unique enzyme, unrelated antigenically to other DNA polymerases. Terminal transferases from different animal species share antigenic determinants, but the cross-reactions are fairly weak. Only tissues that have previously shown terminal transferase by enzymatic assay, specifically bone marrow and thymus (1, 6) have detectable antigenicity of terminal transferase by competition radioimmunoassay.

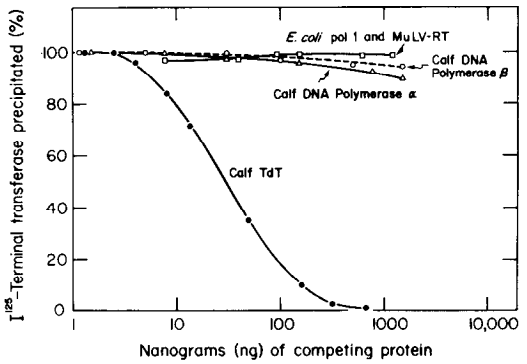

FIG. 9. Determination of antiserum cross-reactivity with other DNA polymerases by competition radioimmunoassay. The preparation of calf DNA polymerase α containing 10 to 30% enzyme protein had a specific activity of 2400 units/mg. The preparation of calf DNA polymerase β containing 50 to 60% enzyme protein had a specific activity of 110,000 units/mg. *Escherichia coli* DNA polymerase I (*E. coli pol 1*) and reverse transcriptase of Moloney leukemia virus (*MuLV-RT*) were homogeneous enzymes. The final dilution of the antiserum was 1/1000 which precipitated 48% of the input ^{125}I-calf terminal transferase (*TdT*) (6,000 cpm).

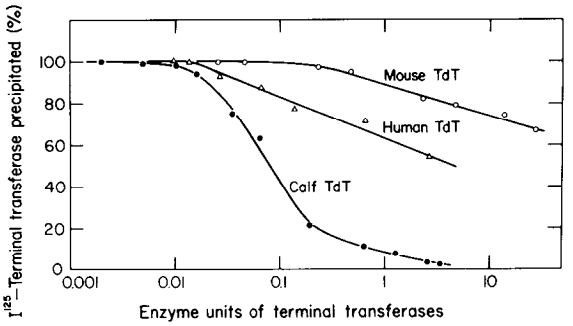

FIG. 10. Antiserum cross-reactivity with heterologous terminal transferase. Human and murine terminal transferase were partially purified as described under "Materials." The amounts of enzyme added were determined from their specific activity. Final concentration of antiserum was 1/1000 which precipitated 45% of the input ^{125}I-terminal transferase (*TdT*) (6,500 cpm).

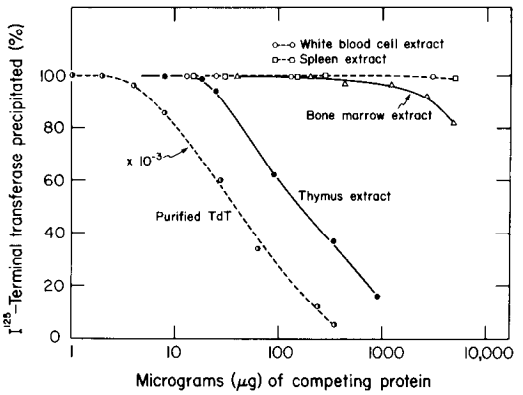

FIG. 11. Tissue distribution of calf terminal transferase. Preparation of cellular extracts was as described under "Methods." Details of the assay were the same as in Fig. 10. Other negative tissues included lymph nodes, muscle, liver, and kidney.

The antigenic uniqueness of terminal transferase relative to other types of DNA polymerases strengthens the concept that terminal transferase is an individual enzyme and not a subunit of a known DNA polymerase. Previous work showing that a rabbit antiserum against calf DNA polymerase α would not inhibit calf terminal transferase activity (17) also indicated the uniqueness of the enzyme. Furthermore, the restricted tissue distribution of terminal transferase, first recognized by Chang (18), argues against its being a subunit of a widely distributed enzyme such as DNA polymerase α, β, or γ.

Using the mouse antisera and ^{125}I-labeled pure enzyme a radioimmunoassay for calf terminal transferase was developed that could detect less than 5 ng of the enyme (Fig. 8). This level of sensitivity is comparable with that of radioimmunoassays used to quantitate reverse transcriptase and other viral proteins (13, 19, 20). With this assay, antigens related to terminal transferase could only be detected in the thymus and bone marrow cells. The level of the enzyme in the thymus is about 280 ng of terminal transferase/mg of soluble protein, which is about 3.5 times higher than the minimum value estimated by Chang and Bollum (2). On the basis of recovery of enzymatic activity, they determined that there is a minimum of 83.5 ng of terminal transferase/mg of protein in the calf thymus. The difference between their estimation and ours is probably a result of the different methods used. The level of terminal transferase in the calf bone marrow is about 100-fold lower than in the thymus. The magnitude of this difference between marrow and thymus is greater than previously found in the mouse by enzymatic assay (5). No molecules related to terminal transferase were found in the spleen, lymph nodes, liver, muscle, kidney, and white blood cells from peripheral blood. The limit of sensitivity of their detection was about 2 ng of terminal transferase/mg of protein.

The number of terminal transferase molecules per thymocyte can be estimated from this data. We find about 19.2 mg of soluble protein per 10^9 thymocytes; at 280 ng of terminal transferase/mg of protein, 10^9 thymocytes should therefore have 5.3 μg of terminal transferase. Terminal transferase has a molecular weight of about 36,000 (2), so there are more than 1×10^5 molecules of terminal transferase per thymocyte. If terminal transferase has a role as a somatic mutator (6, 7), or as a modifier of cell DNA in any way, there is a large enough amount of enzyme present to cause very significant modification.

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